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CRITERIA FOR THE SELECTION OF NUCLIDES FOR RADIOIMMUNOTHERAPY

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INTRODUCTION

For a number of years the scientific and medical communities have contemplated the possibility of using radionuclides for therapy in cancer. The use of sealed sources, such as radium needles and capsules, is now commonplace. With the exception of a relatively select number of applications, the hopes for employing unsealed sources are still unrealized. The problem has two components: the first, and the subject of this conference, is the discovery of a proper carrier molecule with which to bring the radionuclide into the vicinity of the cancer; the second involves interactions between the radionuclide and its biological environment, the radiation biology of the decay products. The accurate estimation of absorbed dose requires information about: the antibody - its specificity, immunoreactivity and stability; the biology of the cancer cell - the number of accessible antigenic sites and their affinity, the homogeneity of antibody presentation among the cancer cells, internalization and modulation of the antigen antibody complex, stability of the complex, stability and translocation of the label out of the complex, and the relationship of antigenicity to the cell cycle; the degree of natural immune surveillance; and the microenvironment of the tumor - its vascularity, its vascular permeability, oxygenation, microscopic organization and architecture including the mobility of the cells, their location and accessibility to intralymphatic, intraperitoneal, intracerebral and intramedullary pathways. In addition, certain properties of the radionuclide to be selected must be known: its mode of decay, including the nature of the particulate radiations and their energies; its physical half-life; and its chemistry in relation to the carrier molecule. Given all this, it is conceivable that sometime in the future one could select a radionuclide for the treatment of a specific tumor taking into account the clinical problem; for example, is one treating microscopic or bulk disease, is one attempting a cure or palliation? In these cases a specific prescription could be written much as the surgeon selects the appropriate operation, the chemotherapist the proper reagent, or the radiotherapist the proper radiation field for treatment.

GENERAL PRINCIPLES

In examining the possibilities for therapy, certain radiobiological

1. The radiations from various radionuclides have different levels of linear energy transfer (LET) and radiobiological effectiveness (RBE). Beta particles are of low LET, and the radiobiological response to them is in accordance, i.e., the RBE is near unity with respect to x-rays in terms of energy deposition, and there is a strong oxygen dependence with well oxygenated cells having a greater radiation sensitivity than less well oxygenated ones. Therefore, with an hypoxic tumor core, there is a mixed uncertain biological response to a given radiation dose. Alpha particles are of high LET. They are more biologically effective than beta particles (on an energy-deposited basis), and their tumoricidal effects are much less oxygen dependent.

2. Radiation responses are generally related to the volume of tumor. Because dose-survival curves for cells are exponential, for a given dose the probability of cell eradication is inversely related to the volume, i.e., the number of cancer cells. For example, if one cell is required to maintain a tumor and the radiation dose treats to a survival fraction of 10^{-5}, with 10^4 cells the chance of one cell surviving is one out of ten or 90% theoretical chance of sterilization; with 10^5 cells, the probability of one cell surviving is quite good. (A tumor containing 10^4 cells has a diameter of about 0.3 mm, while a tumor containing 10^5 has a diameter of about 1 mm.) For this reason, large tumors are unlikely to be amenable to cure except at enormous doses, and it is often advantageous to precede radionuclide therapy with a surgical debulking procedure.

3. The doses required to sterilize dividing cells (cancer) are usually less than those required to kill well differentiated ones (thyrotoxicosis), thousands of rads versus tens of thousands of rads. Unlike the treatment of thyrotoxicosis where functioning intermitotic cells must be killed, for the treatment of cancer the object is to keep the cells from further division.

4. The radiation from radionuclides is generally prolonged in time. This means that the calculated dose is less effective than it would have been if instantaneously delivered. It also suggests that repair processes at work during the period of protraction may spare normal cells relative to cancer cells. As has been made clear in other papers, normal tissue tolerance, especially bone marrow tolerance, is often the factor that limits our ability to deliver a tumoricidal dose.

5. Hyperthermic techniques and anoxic cell sensitizers, adapted to external beam radiotherapy so as to even out the effective dose between oxygenated and hypoxic portions of tumors, can also be utilized in radionuclide therapy.

6. The consequences for nontarget tissues must be addressed: a) Acute (short-term) effects on normal proliferating tissues including depression of the bone marrow after intravenous injections and of the gastrointestinal tract after intraperitoneal injections. In these cases we are concerned about hundreds of rads. By fractionating the administered doses, these effects may be ameliorated to some degree. b) Midterm effects on moderately radiation-sensitive tissues that may receive high doses (in the thousands of rads) where the effects appear to be due principally to vascular damage, for example, radiation hepatitis, nephritis, pneumonitis, and chronic gastrointestinal changes. c) Long-term effects with the appearance of second cancers. This concern need only emerge when one begins to see cures or long-term survivors and the use of these procedures in many cancer patients. Even if they appear, they are not likely to be a deterrent to the continued use of an efficacious treatment. For example, assume a bone marrow dose of 100 rads as a consequence of radioimmunotherapy; the probability of leukemia in a long-term survivor is about 1 in 100.

RADIONUCLIDES FOR THERAPY

The properties one looks for in a radionuclide that is to be used for therapy depend to a large degree on its location in relation to the target cells. Different considerations will prevail if the radionuclide is located outside of the cancer cell, on its surface, in its cytoplasm or in its nucleus. Keep in mind that the radiation sensitive region of the cell is the cell nucleus. Based on the above, one can examine three modes of decay: beta decay, alpha decay, and electron capture with the emission of Auger and Coster-Kronig electrons. Each of these types of decay has particles of varying range and hence effective distance as well as differences in relative biological effectiveness.

Beta Decay

Current radionuclide therapy is based exclusively on beta-emitting radioisotopes. Although in theory both positively and negatively charged particles could be employed, in practice only negatrons are used. Positrons have a range of about 1 mm in tissue before they annihilate to form a pair of photons. The possibility of using them as particles for therapy and simultaneously locating them by annihilation photon tomography has not, to our knowledge, been explored.

Negatively charged beta particles are emitted in continuous values of energy up to a maximum and thus have a distribution of ranges. The average energy of an emitted beta particle is approximately one-third of its maximum energy, and the range in mm is approximately the maximum energy in MeV divided by 0.2. Typically, these light charged particles travel in contorted paths during their interaction with matter. Their track structure is probably more of interest to those working in the field of radioimmunotherapy than has previously been appreciated since the relatively uniform irradiation of tissue assumed for beta-particle emitters independent of their microscopic distribution may be substantially incorrect. Evidence from microautoradiography and from miniature dosimeters suggests that there may be considerable heterogeneity at the hundreds, if not tens, of micron range.[1,2]

Some beta-emitting radionuclides are given in Table 1. The list shows the range of energies and distances that can be covered by beta emitters. Of importance to dosimetry is the equilibrium dose rate constant (Δ_1) in gram-rad per microcurie-hour. This value, when given for the particulate radiation only, reflects the localized radiation dose to be expected from a given quantity of any radionuclide when distributed uniformly in a tissue.

Many beta-particle-emitting radionuclides release gamma photons as well. These photons may be used to locate the distribution of the radionuclide and estimate its quantity; they generally do not add significantly to the dose delivered to the target tissue but, unfortunately, may contribute unwanted radiation dose to nontarget tissues. This gamma radiation can add considerably to the whole body and especially the bone marrow component of dose; for example, 100 millicuries of iodine-131 distributed throughout the whole body provides about 60 rads per day. For radionuclides that emit beta particles of high energy only, the accompanying bremsstrahlung can be used to locate the radionuclide in the body.

In order to gain some appreciation of these delivered doses, we have taken the equilibrium dose rate constants for iodine-131 and yttrium-90 and combined them with pharmacokinetic data provided by Dr. Gerald DeNardo in his paper.[3] Assume that one desires to treat a 2-cm diameter nodular tumor with either a radioiodinated or radioyttriated monoclonal antibody. Assume further that the yttrium labeled antibody conjugate behaves like an indium

Table 1. Beta-Emitting Isotopes for Radiotherapy

Isotope	Half-life	E_β-(max) (keV)	Range (max)[a] (mm)	Δ_i[b] (g-rad/μCi-h)
^{169}Er	9.4 d	350	1.0	0.22
^{121}Sn	27.1 h	380	1.2	0.24
^{67}Cu	61.9 h	580	2.2	0.33
^{131}I	8.0 d	610	2.4	0.40
^{127}Te	9.4 h	700	2.9	0.48
^{83}Br	2.4 h	920	4.2	0.68
^{143}Pr	13.6 d	940	4.2	0.67*
^{198}Au	2.7 d	960	4.4	0.69
^{109}Pd	13.5 h	1030	4.8	0.93
^{186}Re	90.6 h	1070	5.0	0.73
^{165}Dy	2.3 h	1290	6.4	0.95
^{32}P	14.3 d	1710	8.7	1.48
^{188}Re	17.0 h	2120	11.0	1.66
^{142}Pr	19.1 h	2160	11.3	1.72*
^{90}Y	64.1 h	2280	12.0	1.99

a. Calculated using $E = 5.9\ (R + 0.007)^{0.565} + 0.00413\ R^{1.33} - 0.367$

Where R = range in μm
E = maximum β^- energy in keV
See A. Cole, Radiat Res. 38:7 (1969).

b. Δ_i ≡ average equilibrium dose rate constant for beta radiation. All values obtained from ICRP Publication 38; exceptions: * values from NCRP Report 58.

labeled one. In the case of iodine-131 labeled antibody, assume that the nodule takes up 1% of the administered dose and retains it indefinitely so that only physical decay (half-life of 8 days) need be taken into account. The remainder of the administered dose is retained uniformly in the body with an effective half-life of one day. For yttrium-90, again assume that 1% of the administered dose is taken up by the nodule and retained; in this case, the physical half-life is 64 hours. The remainder of the administered activity is retained uniformly in the body with a biological half-life of 7 days, giving an effective half-life of 46 hours. Under these circumstances, 50 millicuries of iodine-131 will deliver 13,500 rads to the tumor nodule and approximately 42 rads to the whole body. In the case of yttrium-90, 31 millicuries will deliver 13,500 rads to the tumor and 82 rads to the whole body. Less yttrium-90 than iodine-131 is required to deliver the same dose to the tumor because of the higher energy (and consequently higher equilibrium dose rate constant) associated with its beta decay. On the other hand, the longer whole body retention for yttrium assumed in this instance makes for a larger whole body dose. As one can see, the dosimetry of these internal emitters depends not only upon the physical properties of the radionuclide but upon the pharmacokinetics and distribution of the labeled antibody as well.

Alpha Particles

Alpha particles are helium nuclei that travel in straight lines with a deposition of considerable energy along their tracks (about 100 keV per micron for usable nuclides). Alpha-emitting radionuclides would have some advantages for therapy. The high LET radiations have correspondingly high

RBE and little oxygen dependence. Moreover, relatively few decays are needed to inactivate the cell. Because the range is relatively short (5-10 cell diameters), it is possible to confine the radiation to tumor cells with high therapeutic ratio (radiation of cancer cells with considerable sparing of normal cells). A disadvantage of alpha-particle-emitting radionuclides is that they require most cancer cells or their nearest neighbors to be labeled in order for all cells to be irradiated. Moreover, the deposition of energy in noncancer cells also is of high RBE and increases the likelihood of tissue damage and second cancers.

Two radionuclides have been suggested for therapy: astatine-211 and bismuth-212. More recently, Bigler has suggested the possible use of fermium-255.[4] Astatine-211 has a half-life of 7.2 hours, and an average alpha particle energy of 6.8 MeV with a particle range of approximately 65 microns. Bismuth-212 has a half-life of 1.1 hours and an alpha particle energy of 5.6 MeV with a range of 54 microns. The half-life of fermium-255 which is 20 hours may be the most favorable. Astatine, the heaviest halogen, has a chemistry that resembles iodine in some ways, and bismuth behaves somewhat like lead.

Although bismuth-212 labeled monoclonal antibodies have been used experimentally to destroy some immunocompetent cells, the radiation biology of astatine-211 has been worked out in somewhat more detail. In vitro dose-survival studies of cells in suspension have shown a monoexponential survival curve (Figure 1) with a D_o of 29 rads; for cells in monolayer, the D_o is 48 rads. Modeling from these survival curves indicates that approximately 1.5 nuclear traversals deliver a mean lethal dose.[5]

When the radionuclide can be confined to the tumor cell compartment, alpha-particle-emitting radionuclides have been demonstrated to be effective in experimental cancer treatment. An astatine colloid can be prepared from tellurium particles (2-25 micron diameter) and, when given to mice implanted with ovarian ascites tumor cells, has been shown both to prolong survival and to result in cures.[6,7] Indeed, when compared with other radionuclide-containing colloids a 50% increase in median survival has been found with 15 microcuries of astatine-211, 100 microcuries of phosphorus-32, 200 microcuries of yttrium-90 and 5 millicuries of dysprosium-165. No cures have

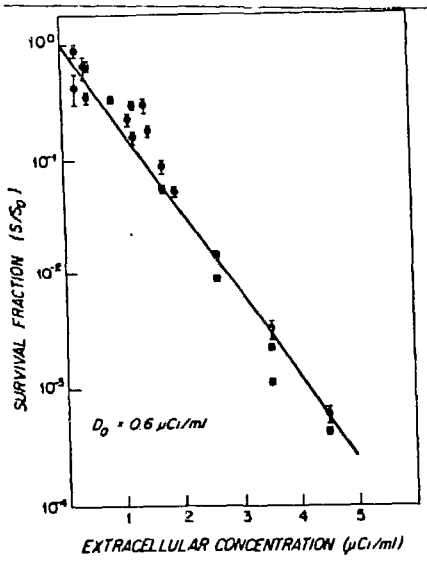

Fig. 1. Dose-survival response of V79 cells in suspension exposed to various concentrations of ^{211}At. Reprinted with permission from Radiat Res. 105:27 (1986).

been seen with any of the beta emitters under circumstances in which cures have been obtained with the optimal dose of astatine-211 (Figure 2).

Electron Capture with Auger and Other Low-Energy-Electron Emission

The response of a cell to the decay of an Auger emitter depends on the cellular localization and concentration of the radionuclide. Two different effects can be described. The extreme radiotoxicity associated with the Auger effect is observed when a shower of Auger and Coster-Kronig electrons with energies ranging from a few to several hundred electron volts is emitted by the radionuclide in the vicinity of nuclear DNA. As a result, extremely high localized doses are produced in microscopic volumes 5 to 10 nanometers in diameter. It has been shown that when the radionuclide is incorporated into one of the pyrimidine bases such high specific ionization results in multiple strand breaks close to the site of decay in DNA and oligonucleotides.[8] In addition, the decay of iodine-125 when part of a pyrimidine base results in the disintegration of the pyrimidine ring.[9] Such molecular lesions would be expected to have profound biological effects, and these have been shown in a number of instances. For example, dose-survival curves for cells labeled with iodine-125 iododeoxyuridine are high LET-like in their appearance with no shoulder on the survival curve and a very steep slope.[10] Similar observations are made when chromosomal aberration frequencies are measured, with iodine-125 being much more effective than iodine-131. These results are observed with other Auger-emitting radionuclides, such as iodine-123[11] and bromium-77,[12] when incorporated into the nuclear materials as halogenated pyrimidine nucleosides. Thus, when these radionuclides are deposited in the cell nucleus, there is a high LET-like radiobiological response with relatively few decays required to inactivate a cell.

On the other hand, when an Auger-electron emitter is present in large quantities in the cytoplasm or on the surface of target cells, the longer-range but still relatively low-energy electrons can still reach the nucleus and produce cell death, albeit with a lesser efficiency. This is demonstrated by the dose-survival curve of cultured cells labeled with iododi-

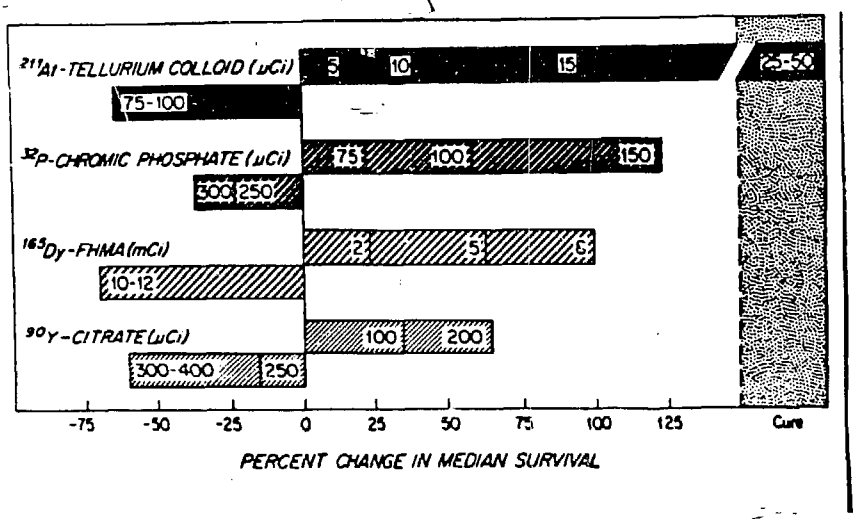

Fig. 2. Radiocolloid therapy on experimental malignant ascites in mice, expressed as the percentage of change in median survival. Reprinted with permission from Int J Radiat Oncol Biol Phys.,10, W. D. Bloomer, W. H. McLaughlin, R. M. Lambrecht, R. W. Atcher, S. Mirzadeh, J. L. Madara, R. A. Milius, M. R. Zalutsky, S. J. Adelstein, and A. P. Wolf, ^{211}At radiocolloid therapy: Further observations and comparison with radiocolloids of ^{32}P, ^{165}Dy, and ^{90}Y, copyright 1984, Pergamon Press, Ltd.

hydrorhodamine, a dye that is concentrated in the cytoplasm.[13] A comparison of the dose-survival curve of cells incubated with iodine-125 labeled iododihydrorhodamine with that of cells incubated with iodine-125 labeled iododeoxyuridine (incorporated into the nuclear material) is shown in Figure 3. Unlike the curve for iododeoxyuridine, the survival curve following the cytoplasmic incorporation of iododihydrorhodamine has a distinct shoulder and a shallower slope resembling that found with iodine-131 labeled iododeoxyuridine.

When other radionuclides that decay by electron capture are concentrated by cells, a good portion of the radiation dose received by the cell is from its own intracellular radioactivity. This is in contrast to the usual beta-emitting radionuclide where the principal sources of radiation exposure are radiations from other cells and from the extracellular fluid. For example, the monovalent cations potassium-43 and rubidium-86, which decay by beta minus emissions, and thallium-201, which is an Auger-electron emitter, are concentrated by mammalian cells. It has been shown that thallium-201 is much more toxic to dispersed cells than potassium-43 and rubidium-86 on a per picocurie basis. The main source of radiation for the thallium-containing cells is their own intracellular radioactivity. The exclusion of thallium by the use of ouabain or low temperatures reduces the radiation toxicity, a phenomenon that is not observed when the other cations are restricted from entering the cells.[14]

It is possible that such concentration effects could be achieved by the use of monoclonal antibodies, especially with those that become internalized within cells. Table 2 shows the results of an experiment[15] with three monoclonal antibodies to RINm5F cells: A2B5, A1D2 and 3G5. The cells have been incubated in vitro with each monoclonal antibody. All three antibodies react initially with the cell membrane, as shown by the fluorescence which decays with a half-life of approximately one hour. Following 24 hours in antibody-free medium, the radioactivity associated with A2B5 is principally retained, while with A1D2 about 50% of the radioactivity remains and with 3G5 only about 10%. Since the membrane fluorescence has virtually disappeared by the end of three hours, it seems reasonable to conclude that the

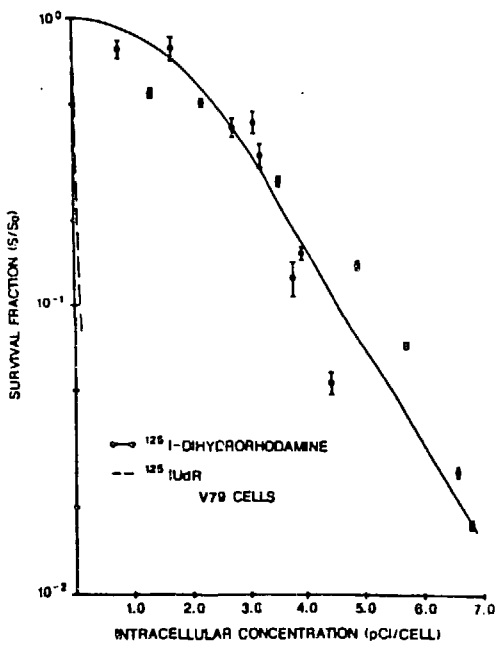

Fig. 3. Comparison of the survival of V79 cells following nuclear (^{125}IUdR) or cytoplasmic (^{125}I-dihydrorhodamine) incorporation of ^{125}I.

Table 2. Monoclonal Antibody Binding and Retention by RINm5F Cells In Vitro

Time (hr)	Cell Membrane Fluorescence	Cell Associated CPM ± SD (% retained) A2B5	A1D2	3G5
0.0	++++	7276 ± 1127	866 ± 31	2109 ± 110
1.0	++	---	466 ± 101(53.8)	971 ± 65(46.0)
3.0	±	6949 ± 1068(96)	490 ± 23(56.6)	590 ± 80(28.0)
24.0		5922 ± 1302(81)	595 ± 15(68.7)	200 ± 50(10)

retained radioactivity is within the cell. Under these circumstances, labeling with radionuclides that emit electrons of short range (several microns) could have a high therapeutic ratio, the decays being especially toxic to cells in which the radionuclide is internalized and much less toxic to cells from which it is totally excluded.

SUMMARY AND CONCLUSIONS

Many factors need to be taken into account and many problems resolved if radioimmunotherapy is to become a commonplace reality. Among these are the radiobiological aspects. For beta-emitting radionuclides, the two physical features of importance are half-life and energy, with the latter determining the range. These features will have to be matched to the pharmacokinetics of the carrier and the distribution of the radionuclide, both macroscopically and microscopically. Alpha-particle emitters could be considered for cells that are readily accessible to the labeled antibody and for populations which uniformly and constantly display the targeted antigen or idiotype, e.g., trafficking cells such as T or B lymphocytes. For cells that concentrate the radioactive label (by internalization, transchelation, etc.), the use of low-energy electrons should be examined. If the radionuclide is translocated to the nucleus, the Auger effect can be particularly lethal because of the high LET-like biological response.

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